spectra can be attributed to transitions involving these two electronic levels. The value of the separation of the vibrational structure of 1200-1300 cm^{-1} also supports this assignment, since the C-O stretching vibration mode occurs at ca. 1250 cm^{-1}. We suggest that the presence of this band at ca. 10000-13000 cm^{-1} is diagnostic of the semiquinone character of a coordinated dioxolene ligand. Although this criterion cannot be exploited in all of the cases, due to the possible presence of other more intense bands, it is extremely useful and simple to apply. A scan of the literature showed for instance that in $M_4(DTBSQ)_8$ complexes (M = Co, Ni, Mn),[14] where the semiquinone nature of the ligands was established by X-ray crystal structure, bands are present at ca 13000 cm^{-1} thus confirming our assignment.

The determination of the origin of the pattern of bands occurring in the UV region is not straightforward. On the basis of the relative intensities and the values of the dissymmetry factors, we assign the bands at highest energy to the same transitions occurring in the catecholate derivatives, i.e. $3b_1 \rightarrow 4b_1$ and $3b_1 \rightarrow 3a_2$. The band with a vibronic progression observed in the near-UV region is tentatively assigned to the $2a_2 \rightarrow 3b_1$ transition, which has $\pi \rightarrow \pi^*$ character, on the basis of its absence in the spectra of catecholate complexes and of its intensity.

Finally, the broad band in the visible region of the CD spectrum is tentatively assigned to the $n \rightarrow \pi^*$ electric dipole forbidden and magnetic dipole allowed $7b_2 \rightarrow 3b_1$ transition.

We assign to charge-transfer transitions the bands observed at 21200 and 19200 cm^{-1} for the nickel(II)-DTBSQ and -TCSQ derivatives, respectively. These bands show a red shift on passing from the DTBSQ to the TCSQ derivative, consistent with a MLCT character of the electronic excitation. It appears reasonable to assign this transition to the spin-allowed $t_{2g} \rightarrow \pi^*$ quartet transition, taking into account its intensity and dissymmetry factor. This assignment requires a difference of optical electronegativity between the nickel(II) and the semiquinone ligand of about 1.[27] Other assignments involving doublet terms appear less probable, taking into account the energy difference between the ground state and the first doublet excited states.

The proposed assignment for both catecholates and semiquinonates agrees well with the observed chemical properties of these systems. Oxidation of divalent metal-catecholate adducts can occur either on the metal, yielding metal(III) catecholates, or on the ligand, yielding metal(II) semiquinonates. We have found[19] that for CTH complexes the former process occurs with chromium-, manganese-, iron-, and cobalt-catecholate adducts, in agreement with the results of previous investigations on other chemically related systems.[1,2] Nickel(II) represents the crossover and together with copper(II) and zinc(II) forms stable semiquinonate adducts.

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Registry No. *SS*-CTH, 53187-81-8; α-Ni(*SS*-CTH)$(ClO_4)_2$, 80531-86-8; Zn(*SS*-CTH)$(PF_6)_2$, 119565-86-5; Ni(*SS*-CTH)(DTBSQ)ClO_4, 119565-88-7; Ni(*SS*-CTH)(TCCat), 119480-47-6; Ni(*SS*-CTH)(TCSQ)ClO_4, 119480-49-8; Zn(*SS*-CTH)(DTBSQ)PF_6, 119480-51-2; Zn(*SS*-CTH)(TCCat), 119480-52-3; Ni(*SS*-CTH)(DTBSQ)PF_6, 119615-74-6; 3,5-di-*tert*-butyl-1,2-dihydroxybenzene, 1020-31-1.

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Stability and Structure of Xanthosine-Metal Ion Complexes in Aqueous Solution, Together with Intramolecular Adenosine-Metal Ion Equilibria

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The stability constants of the 1:1 complexes formed between Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Cu(2,2'-bipyridyl)$^{2+}$, or Cu(1,10-phenanthroline)$^{2+}$ and xanthosine (Xao) were determined by potentiometric pH titration in aqueous solution (I = 0.1 ($NaNO_3$); 25 °C). Xanthosine is deprotonated at N-1 with pK^H_{Xao} = 5.47, and in accord herewith $M(Xao\text{-}H)^+$ complexes are formed. The anion $(Xao\text{-}H)^-$ displays a dichotomy between metal ion binding at N-1 and binding at N-7. The ratios for the metal ion distribution between these two sites are estimated: Cu^{2+}, like the proton, strongly favors binding at N-1; Ni^{2+} also binds about 70% at this site; Mn^{2+} and Cd^{2+} prefer N-7 binding by about 75%; Co^{2+} and Zn^{2+} are more equally distributed between the two sites. In connection with these structural evaluations previous conclusions regarding metal ion binding in M(adenosine)$^{2+}$ complexes are reconsidered; it is suggested that the amino group next to N-1 in adenosine gives rise to steric hindrance in the case of N-1 metal ion coordination. When this and some other effects are taken into account, it is concluded that Ni^{2+}, Cu^{2+}, and Zn^{2+} coordinate to adenosine preferably via the N-7 site. From the relationships obtained for N-1 and N-7 coordination between the log K stability constants and the ligand pK_a values, other stability constants may be estimated, provided the ligand pK_a is known. This procedure is applied to calculate with $pK^H_{H(Xao)}$ = 0.74 and the indicated relationships the stability constants of the complexes formed between Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+} and neutral xanthosine; the resulting constants are compared with some experimentally obtained estimations. Metal ion coordination occurs in these $M(Xao)^{2+}$ complexes at the N-7 site. Finally it is emphasized that the indicated relationships may be used to judge the validity of published stability constants for nucleoside-metal ion complexes; this is an important aspect, as here many pitfalls are buried.

Enzymes utilizing nucleotides as substrates are in general also metal ion dependent (e.g. ref 2-4). This explains why great efforts are being made to understand and to quantify the interactions between metal ions and nucleotides in the solid state (e.g. ref 5 and 6) and in solution (e.g. ref 7-11).

Our own efforts are presently concentrated on the stability and structure of the complexes between nucleoside monophosphates and the divalent alkaline-earth or 3d transition-metal ions.[12,13]

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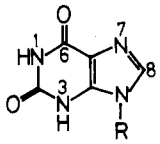

Figure 1. Chemical structure of xanthosine (Xao).

As xanthine derivatives are important for the metabolism of purine nucleotides,[14] we are attempting to include in our studies xanthosine 5′-monophosphate (XMP^{2-}). During this attempt it became evident that detailed knowledge of the coordinating properties of xanthosine (Xao) is compulsory.

Available information[15-21] on the stability of xanthosine–metal ion complexes contrasted with our own experience. This forced us to study the acid–base properties of xanthosine (Figure 1) and to determine the stabilities of its corresponding 1:1 complexes with Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+}. The results also include data on mixed-ligand Cu^{2+} complexes with Xao and 2,2′-bipyridyl (bpy) or 1,10-phenanthroline (phen) (Arm = bpy, phen). In addition we present an attempt to evaluate the metal ion affinity of the N-1 site versus that of the N-7 site in the monoanion of xanthosine $(Xao-H)^-$; with regard to the structure of $M(Xao-H)^+$ complexes such considerations are important. The reasonings on the N-1/N-7 dichotomy are also applied to adenosine complexes and discussed in relation to previous considerations[22] of this problem.

Experimental Section

Materials. Xanthosine was from Sigma Chemical Co., St. Louis, MO. Cytidine, 2,2′-bipyridyl, 1,10-phenanthroline hydrate, the disodium salt of ethylenediamine-*N,N,N′,N′*-tetraacetic acid (Na_2H_2EDTA), potassium hydrogen phthalate, HNO_3, NaOH (Titrisol), and the nitrate salts of Na^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} (all pro analysi) were from Merck AG, Darmstadt, FRG. All solutions were prepared with distilled CO_2-free water.

The exact concentrations of the nucleoside solutions were measured by titrations with NaOH. Its titer was determined with potassium hydrogen phthalate, and the concentrations of the stock solutions of the metal ions were established with EDTA.

Potentiometric pH Titrations. The pH titrations were carried out with a Metrohm E536 potentiograph, E655 dosimat, and 6.0202.100 (JC) macro glass electrodes. The buffer solutions (pH 4.64, 7.00, and 9.00) used for calibration were also from Metrohm AG, Herisau, Switzerland. The direct pH meter readings were used in the calculations of the acidity constants. These were done with a Hewlett-Packard 9825A calculator, connected with a 7470A plotter and an 82905B printer. The calculations of the stability constants were carried out with a Hewlett-Packard Vectra 60PC calculator connected with a quietjet plus printer.

The differences between $pK^H_{H(Xao)}$ and pK^H_{Xao} or pK^H_{Xao} and $pK^H_{(Xao-H)}$ are large (>4.5 log units); hence, the buffer regions between $H(Xao)^+$, Xao, and $(Xao-H)^-$ are not overlapping, allowing an independent determination of the acidity constants. The upper and lower limits for $pK^H_{H(Xao)}$ and $pK^H_{(Xao-H)}$, respectively, and some values for pK^H_{Xao} were determined by titrating 20 mL of aqueous 15 mM HNO_3 (I = 0.1 ($NaNO_3$); 25 °C) in the presence and absence of 4.5 mM xanthosine under N_2 with 2 mL of 0.2 M NaOH and by using the differences in NaOH consumption between such a pair of titrations for the calculations. Most of the values for K^H_{Xao} were determined by titrating 50 mL of aqueous 0.3 mM HNO_3 and $NaNO_3$ (I = 0.1; 25 °C) in the presence and absence of 0.56 mM Xao under N_2 with 1 mL of 0.05 M NaOH. Values for K^H_{Xao} were calculated from 17 independent pairs of such titrations by taking into account the species H^+, Xao, and $(Xao-H)^-$ with the Hewlett-Packard 9825A calculator by a curve-fit procedure using a Newton–Gauss nonlinear-least-squares program within the pH range determined by about 3% and 97% neutralization for the equilibrium Xao/$(Xao-H)^-$. The upper limit for $K^H_{H(Xao)}$ and the lower one for $K^H_{(Xao-H)}$ were correspondingly determined from two and seven independent pairs of titrations, respectively, by extending the pH range as far as possible into the appropriate direction.

The stability constants $K^M_{M(Xao)}$ and $K^M_{M(Xao-H)}$ (or $K^{Cu(Arm)}_{Cu(Arm)(Xao)}$ and $K^{Cu(Arm)}_{Cu(Arm)(Xao-H)}$) of $M(Xao)^{2+}$ and $M(Xao-H)^+$ (or $Cu(Arm)(Xao)^{2+}$ and $Cu(Arm)(Xao-H)^+$) were determined under the same conditions as for most of the values of the acidity constant, K^H_{Xao}, i.e. with [Xao] = 0.56 mM, but $NaNO_3$ was partially or fully replaced by $M(NO_3)_2$ (I = 0.1; 25 °C). For all M^{2+} systems, including Cu^{2+}/Arm (1:1), titrations were made with $[M^{2+}]$ = 33.3 mM (i.e., Xao:M^{2+} = 1:59.5); under other conditions for Mn^{2+} and Zn^{2+} $M(NO_3)_2$ was 30 mM (Xao:M^{2+} = 1:53.6), for Co^{2+}, Ni^{2+}, and Cd^{2+} $M(NO_3)_2$ was 23.3 mM (Xao:M^{2+} = 1:41.6), and for Cu^{2+} and Cu^{2+}/Arm (1:1) $Cu(NO_3)_2$ was 13.3 mM and also 6.67 mM (Xao:Cu^{2+} = 1:23.8 and also 1:11.9).

Some preliminary experiments were also carried out with cytidine; the experimental conditions were exactly as for xanthosine except $[HNO_3]$ = 0.9 mM, [Cyd] = 0.6 mM, and in the presence of Ni^{2+} or Cu^{2+} $[M(NO_3)_2]$ = 33.3 mM.

Calculation of the Stability Constants of the Complexes. The stability constants $K^M_{M(Xao-H)}$ were computed for each pair of titrations by taking into account the species H^+, Xao, $(Xao-H)^-$, M^{2+}, and $M(Xao-H)^+$.[23] Throughout, the data were collected (every 0.1 pH unit) from about 5% complex formation to a neutralization degree of about 85% or to the beginning of the hydrolysis of M^{2+}(aq), which was evident from the titrations without xanthosine: e.g., for the Cu^{2+} system (with the highest concentration) the pH range 3.5–4.4 was evaluated and for the Mn^{2+} system the pH range evaluated was 5.1–5.8. In the case of the xanthosine systems with Co^{2+}, Ni^{2+}, Cu^{2+}, and Cd^{2+} the experimental data could be slightly better satisfied with a curve-fitting procedure[24] taking into account in addition to the mentioned species also $M(Xao)^{2+}$. However, the results obtained for $K^M_{M(Xao)}$ are only estimates as the formation degree of $M(Xao)^{2+}$ is mostly below 10%; even under the most favorable conditions with $[Cu^{2+}]$ = 33.3 mM and [Xao] = 0.56 mM only a formation degree of 15% is reached for $Cu(Xao)^{2+}$.

In the pH range (3.5–5.1) used for the calculation of the stability constants of the ternary $Cu(Arm)(Xao)^{2+}$ and $Cu(Arm)(Xao-H)^+$ species, complex formation between Cu^{2+} and bpy or phen is already complete due to the great stability of $Cu(Arm)^{2+}$;[25] this was also evident from the identity of the titration curves obtained from a pair of solutions, one that contained HNO_3 only and another that contained Cu^{2+}/Arm in addition. Hence, in the calculations only complex formation between $Cu(Arm)^{2+}$ and Xao or $(Xao-H)^-$ had to be considered,[26] and each of these systems could be treated as a binary one (see above) by considering the species H^+, Xao, $(Xao-H)^-$, $Cu(Arm)^{2+}$, $Cu(Arm)(Xao)^{2+}$, and $Cu(Arm)(Xao-H)^+$. However, the results obtained for $K^{Cu(Arm)}_{Cu(Arm)(Xao)}$ are again only estimates due to the low formation degree of $Cu(Arm)(Xao)^{2+}$.

For the determinations of the stability constants always at least five independent pairs of titrations were made. The values calculated individually for $\log K^M_{M(Xao-H)}$ or $\log K^{Cu(Arm)}_{Cu(Arm)(Xao-H)}$ showed no dependence on pH or (where applicable) on the excess of M^{2+} or $Cu(Arm)^{2+}$. This means that there was no hint of the formation of $M_2(Xao-H)^{3+}$, which could be thought to exist due to the presence of the N-1 and N-7 binding site in $(Xao-H)^-$. However, this is understandable: the constants $K^M_{M(Xao)}$ of $M(Xao)^{2+}$ are already small and for $K^{M(Xao-H)}_{M_2(Xao-H)}$ even smaller values are expected.

Spectrophotometric Measurements. The acidity constant $pK^H_{H(Xao)}$ of $H(Xao)^+$ was determined by spectrophotometry. The UV spectra ([Xao] = 0.07 mM) were recorded on a Cary 219 instrument in aqueous solutions at 25 °C and I = 0.5 ($NaClO_4$) with 1-cm quartz cells. An example of an experimental series is shown in Figure 2 (vide infra); in the two experiments giving the data points at pH −0.97 and −0.76 the ionic strength is >0.5 M, but these points mainly determine only the limiting value for the absorption of $H(Xao)^+$ and not the position of the buffer region. The pH of the other solutions (see Figure 2) was adjusted with

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Table I. Negative Logarithms of the Acidity Constants (Eq 1-3) Determined in Aqueous Solution for Monoprotonated Xanthosine, Together with Earlier Data from the Literature[a]

ionic strength (I)	T, °C	$pK^{H}_{H(Xao)}$	pK^{H}_{Xao}	$pK^{H}_{(Xao-H)}$	ref
0.1 ($NaNO_3$)[c]	25	<0.9	5.47 ± 0.03	>12.0	*b*
0.5 ($NaClO_4$)[d]	25	0.74 ± 0.06			*b*
e	20	<2.5	5.67	>11	15
0	25		5.67	12.00	32
0.1 (KNO_3)	25		5.58	9.87	16, 17
0.1 ($NaNO_3$)[e,f]	25	2.11 ± 0.04[f]	9.22 ± 0.01[f]	>12.0[f]	33

[a] See text. For comparison, the corresponding acidity constants are also given for monoprotonated guanosine (see footnote *f*). [b] This work. The errors given are *3 times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. With regard to the sites from which the protons are released, see text in section 1. [c] Determined by potentiometric pH titration. [d] From spectrophotometric experiments (see also Figure 2). [e] Measured probably at a low and natural ionic strength with [Xao] = 0.002 M.[15] [f] Values for guanosine.

$HClO_4$ and measured with a Metrohm 605 digital pH meter using a Metrohm 6.0202.100 (JC) glass electrode. Each solution was individually prepared and measured within less than 5 min to prevent hydrolysis of xanthosine;[27] at pH 0.6 a change in absorption is observable only after 30 min.

The spectrophotometric data were analyzed with the Hewlett-Packard 9825A calculator analogously to the potentiometric data. The final result given in Table I (vide infra) is the average of five independent series of experiments.

Results and Discussion

All experiments were carried out under conditions where the self-association of xanthosine is expected to be negligible, due to previous experience with related nucleosides.[28-30] Most of the potentiometric pH titrations for the determination of the acidity constants of xanthosine and all of the titrations for the stability constants of the binary $M(Xao)^{2+}$ or $M(Xao-H)^{+}$ and the ternary $Cu(Arm)(Xao)^{2+}$ or $Cu(Arm)(Xao-H)^{+}$ complexes were made with [Xao] = 0.56 mM (see Experimental Section). In the spectrophotometric experiments even a lower Xao concentration was used.

1. Acidity Constants of Monoprotonated Xanthosine. Xanthosine (see Figure 1) has only a single basic site, i.e. N-7, which may undergo protonation by forming $H(Xao)^{+}$; the corresponding site also exists in inosine and guanosine moieties.[22,31] The affinity of N-7 in xanthosine for protons is rather low, and a pK_a value could therefore only be determined by spectrophotometric experiments; an example for such a series is shown in Figure 2. Neutral xanthosine releases a proton from the H(N-1) site, a site attribution[32] in accordance with that of related nucleosides or nucleotides.[22,28] In this latter reaction, $(Xao-H)^{-}$ is formed; the corresponding pK_a is easily determined by potentiometric pH titrations. Finally, there are indications that a further proton may be released, most probably from the ribose residue.[32] Hence, overall three protonation equilibria have to be considered:

$$H(Xao)^{+} \rightleftharpoons Xao + H^{+} \quad (1a)$$

$$K^{H}_{H(Xao)} = [Xao][H^{+}]/[H(Xao)^{+}] \quad (1b)$$

$$Xao \rightleftharpoons (Xao-H)^{-} + H^{+} \quad (2a)$$

$$K^{H}_{Xao} = [(Xao-H)^{-}][H^{+}]/[Xao] \quad (2b)$$

$$(Xao-H)^{-} \rightleftharpoons (Xao-2H)^{2-} + H^{+} \quad (3a)$$

$$K^{H}_{(Xao-H)} = [(Xao-2H)^{2-}][H^{+}]/[(Xao-H)^{-}] \quad (3b)$$

The results are summarized in Table I, together with some earlier data[15-17,32] and the corresponding acidity constants for the related nucleoside guanosine.[33]

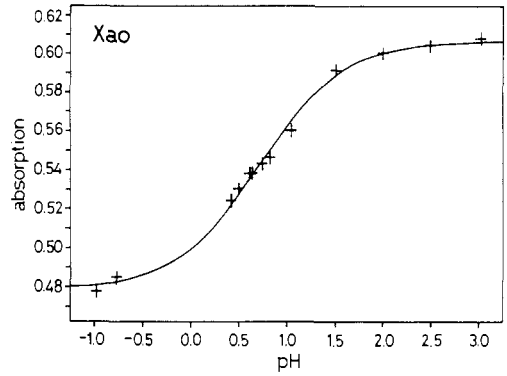

Figure 2. Evaluation of the dependence of the UV absorption of xanthosine at 239 nm on pH in aqueous solution (measured in 1-cm cells; [Xao] = 7 × 10^{-5} M; I = 0.5 ($NaClO_4$); 25 °C) by plotting the absorption versus pH. The solid curve represents the computer-calculated best fits of the experimental data points at pH −0.97 (9.3 M $HClO_4$), −0.76 (5.8 M $HClO_4$), 0.42, 0.50, 0.61 0.64, 0.74, 0.82, 1.04, 1.50, 2.00, 2.49, and 3.01, which leads for this experiment to $pK^{H}_{H(Xao)}$ = 0.72 ± 0.09 (3σ) for the deprotonation of $H(Xao)^{+}$ (see also Experimental Section).

The upper limits given in Table I for the release of the proton from $H(Xao)^{+}$, i.e. for $pK^{H}_{H(Xao)}$ (eq 1), agree with the constant actually measured by spectrophotometry. Considering the different experimental conditions, the agreement of the values for the deprotonation of neutral xanthosine (eq 2), i.e. for pK^{H}_{Xao}, is fair. However, the value of 9.87 given[16,17] for $pK^{H}_{(Xao-H)}$ and attributed to the release of a proton from H(N-3) (Figure 1) is wrong. We have carefully searched for the release of a further proton from $(Xao-H)^{-}$ up to pH 12.0 without success; hence, we must conclude that the release of a further proton either from the H(N-3) site[16,17] or from the ribose moiety[32] occurs only with $pK^{H}_{(Xao-H)} > 12.0$ (eq 3) (Table I).

Finally, one may point out that xanthosine and guanosine are closely related nucleosides as is evident also from their metabolism:[14] guanosine may be transformed into xanthosine by replacing the NH_2 at C-2 with a OH group; tautomerization of this enol into the keto form gives xanthosine (Figure 1). However, this exchange of the substituent at C-2 dramatically alters the properties of the H(N-1) site in the pyrimidine ring: this site is 3.75 log units more acidic in xanthosine (pK^{H}_{Xao} = 5.47) than in guanosine (pK^{H}_{Guo} = 9.22). The effect in the neighboring imidazole ring is less dramatic (Table I) but still remarkable: N-7 is about 1.4 log units less basic in neutral xanthosine than in guanosine. These alterations of the acid-base properties are expected to be reflected in the coordinating qualities not only of the nucleosides but also of the corresponding nucleotides.

2. Stability of Binary $M(Xao)^{2+}$ and $M(Xao-H)^{+}$ Complexes. The experimental data of the potentiometric pH titrations may be completely described by considering equilibria 2, 4, and 5 (see also section 1). The stability constants calculated in the individual

$$M^{2+} + Xao \rightleftharpoons M(Xao)^{2+} \quad (4a)$$

$$K^{M}_{M(Xao)} = [M(Xao)^{2+}]/([M^{2+}][Xao]) \quad (4b)$$

$$M^{2+} + (Xao-H)^{-} \rightleftharpoons M(Xao-H)^{+} \quad (5a)$$

$$K^{M}_{M(Xao-H)} = [M(Xao-H)^{+}]/([M^{2+}][(Xao-H)^{-}]) \quad (5b)$$

experiments show no dependence on pH or on the excess of the metal ion concentration, if the evaluation is not carried into the pH range where hydroxo complexes form (cf. Experimental Section). It should be emphasized that the experimental data give only estimates of log $K^{M}_{M(Xao)}$ for some of the $M(Xao)^{2+}$ complexes (cf. Experimental Section); these constants will therefore be further considered in section 8. In addition, the experimental

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Table II. Logarithms of the Stability Constants of Binary $M(Xao-H)^+$ (Eq 5) and Ternary $Cu(Arm)(Xao-H)^+$ Complexes (Eq 7), Together with the Estimates Obtained for Some of the Corresponding $M(Xao)^{2+}$ (Eq 4) and $Cu(Arm)(Xao)^{2+}$ Species (Eq 6), As Determined by Potentiometric pH Titrations in Water at 25 °C and I = 0.1 ($NaNO_3$)[a]

M^{2+}	log $K^{M}_{M(Xao)}$[b]	log $K^{M}_{M(Xao-H)}$	Δ log K_{Cu}
Mg^{2+}		<0.6[c]	
Ca^{2+}		<0.6[c]	
Sr^{2+}		<0.6[c]	
Ba^{2+}		<0.6[c]	
Mn^{2+}		0.84 ± 0.05	
Co^{2+}	0.5 ± 0.2	1.65 ± 0.05	
Ni^{2+}	0.7 ± 0.2	2.09 ± 0.05	
Cu^{2+}	0.8 ± 0.3	2.58 ± 0.03	
Zn^{2+}		1.32 ± 0.02	
Cd^{2+}	0.70 ± 0.12	1.96 ± 0.05	
$Cu(bpy)^{2+}$	0.64 ± 0.18	2.49 ± 0.03	−0.09 ± 0.04
$Cu(phen)^{2+}$	0.7 ± 0.3	2.48 ± 0.03	−0.10 ± 0.04

[a] The resulting values for Δ log K_{Cu} (eq 8 and 9) are also listed. The error limits given are *3 times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The values of the error limits for Δ log K_{Cu} were calculated according to the error propagation method of Gauss. [b] See also Table VII. [c] Detailed evaluations of the experimental data indicated that for all four alkaline-earth ions log $K^{M}_{M(Xao-H)} \simeq 0.2 \pm 0.2$.

data also provide no evidence for the formation of $M_2(Xao-H)^{3+}$ complexes; the stability of such species is expected to be very low (see Experimental Section).

The stability constants determined for $M(Xao-H)^+$ complexes (eq 5) and the estimates for some $M(Xao)^{2+}$ species (eq 4; see also section 8) show the usual trends (Table II): Complex stability with the alkaline-earth ions is low. For the divalent 3d metal ions a stability sequence corresponding to the Irving–Williams series[34] is observed; the relatively large stability differences between the $M(Xao-H)^+$ complexes with Cu^{2+} and Mn^{2+} (1.7 log units) or Cu^{2+} and Zn^{2+} (1.3 log units) are characteristic[35] of nitrogen donor ligands (see also section 5).

Unfortunately, the agreement between the present results and previous data is poor: the constants given in ref 15 for the $M(Xao-H)^+$ complexes of Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} are about 0.8–1.2 log units too large. This discrepancy cannot be solely attributed to different experimental conditions (20 °C and I = natural?); instead it appears, on the basis of the experimental part in ref 15, that the hydrolysis of the $M^{2+}(aq)$ ions was neglected. The data of ref 16 and 17 have been determined under conditions (25 °C; I = 0.1 M (KNO_3)) similar to ours, but the agreement is even worse: the logarithms of the stability constants given for the $M(Xao-H)^+$ complexes of Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, and Zn^{2+} are all between 2.22 and 2.48; the corresponding values for the Ni^{2+} and Cu^{2+} complexes are 2.88 and 2.91, respectively. Hence, these previous constants are between about 0.3 and 2.2 log units too large, if compared with the results in Table II. The situation with the constants given in ref 19–21 is similar: for Co^{2+}, Ni^{2+}, and Cu^{2+} log $K^{M}_{M(Xao-H)}$ varies between 2.88 and 3.42 (25 °C; I = 0.1 M (KNO_3)); i.e., these constants are about 0.8–1.2 log units too large (as are those of ref 15). Most probably hydrolysis of $M^{2+}(aq)$ was neglected also in the experiments of ref 19–21. All these previous values in ref 15–17 and 19–21 have to be rejected. That the data of ref 16 and 17 are erroneous is evident already from the fact that the stability constant given for $Ca(Xao-H)^+$ (log K = 2.37) is larger than the one for $Zn(Xao-H)^+$ (log K = 2.26) or for $Co(Xao-H)^+$ (log K = 2.23); for a ligand like xanthosine such properties would be in contrast to all experience.[34,35] This contrast does however not exist; the stability constants listed in Table II agree with experience.

3. Stability of Ternary $Cu(Arm)(Xao)^{2+}$ and $Cu(Arm)(Xao-H)^+$ Complexes. The stability of the mixed-ligand complexes of xanthosine, Cu^{2+}, and an aromatic amine (Arm), i.e. 2,2′-bipyridyl (bpy) or 1,10-phenanthroline (phen), was also determined. The potentiometric pH data can be completely satisfied in a way analogous to that given in section 2 for the binary systems; this means that now equilibria 2, 6, and 7 have to be considered in the calculations. The corresponding constants are listed in Table II; the values for $Cu(Arm)(Xao)^{2+}$ are again only estimates (section 2). For $Cu(bpy)(Xao-H)^+$ log $K^{Cu(bpy)}_{Cu(bpy)(Xao-H)} = 2.36$ has previously been determined,[19] in fair agreement with the present result.

$$Cu(Arm)^{2+} + Xao \rightleftharpoons Cu(Arm)(Xao)^{2+} \quad (6a)$$

$$K^{Cu(Arm)}_{Cu(Arm)(Xao)} = \frac{[Cu(Arm)(Xao)^{2+}]}{[Cu(Arm)^{2+}][Xao]} \quad (6b)$$

$$Cu(Arm)^{2+} + (Xao-H)^- \rightleftharpoons Cu(Arm)(Xao-H)^+ \quad (7a)$$

$$K^{Cu(Arm)}_{Cu(Arm)(Xao-H)} = \frac{[Cu(Arm)(Xao-H)^+]}{[Cu(Arm)^{2+}][(Xao-H)^-]} \quad (7b)$$

The relative stability of ternary complexes toward their binary parent complexes is best quantified by considering equilibrium 8a.[36,37] The corresponding constant defined by eq 8b is calculated

$$Cu(Arm)^{2+} + Cu(Xao-H)^+ \rightleftharpoons Cu(Arm)(Xao-H)^+ + Cu^{2+} \quad (8a)$$

$$10^{\Delta \log K_{Cu}} = \frac{[Cu(Arm)(Xao-H)^+][Cu^{2+}]}{[Cu(Arm)^{2+}][Cu(Xao-H)^+]} \quad (8b)$$

with eq 9. Equilibrium 8a is expected[36-38] to be on its left side

$$\Delta \log K_{Cu} = \log K^{Cu(Arm)}_{Cu(Arm)(Xao-H)} - \log K^{Cu}_{Cu(Xao-H)} = \log K^{Cu(Xao-H)}_{Cu(Xao-H)(Arm)} - \log K^{Cu}_{Cu(Arm)} \quad (9)$$

with negative values for Δ log K_{Cu} due to the general rule that $K^{M}_{M(L)} > K^{M(L)}_{M(L)_2}$. Indeed, statistical considerations[36,39] for the coordination of a bidentate ligand followed by a monodentate (or bidentate) ligand to the tetragonal or Jahn–Teller-distorted octahedral coordination sphere of Cu^{2+} give Δ log $K_{Cu/statist} \simeq -0.5$ (or −0.9).

The constants listed in Table II for Δ log K_{Cu} are slightly negative but are somewhat larger than the statistically expected value. This result appears to be in agreement with the coordination of a negatively charged nitrogen site to $Cu(Arm)^{2+}$, as will be further discussed in section 7. It should be pointed out that no *positive* values for Δ log K_{Cu} are obtained; such an observation is usually made if (i) the ternary complex is formed with heteroaromatic N bases and O-donor ligands[23,36-38] and/or (ii) intramolecular aromatic-ring stacks are formed between suitable ligand parts within the mixed-ligand complex.[36,37,39-41]

On the basis of previous experience with mixed-ligand complexes[23,37,38,42] negative Δ log K_M values (eq 8 and 9) are also expected for other $M^{2+}/Arm/(Xao-H)^-$ systems. This means that the stability of other ternary $M(Arm)(Xao-H)^+$ complexes may be estimated by subtracting 0.2 log unit from the stability constants of the binary complexes in Table II; the resulting estimate is expected to be correct within ±0.2 log unit. However, there is a limit to this procedure: 2,2′-bipyridyl and 1,10-phenanthroline

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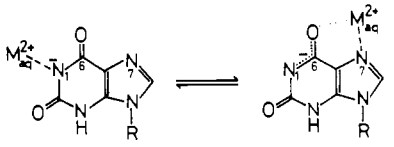
form stacking adducts with purine derivatives,[9,36,43] and therefore formation of (Arm)(Xao) and (Arm)(Xao-H)$^-$ stacks has to be considered. The stability constant for these adducts is expected[9,44] to be on the order of 10; i.e., log $K \approx 1$. Hence, in the ternary systems with Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, and Mn^{2+} (and possibly even Zn^{2+}) the situation is governed by the stability of the mentioned aromatic-ring stacking adducts and *not* by the metal ions, though these may still coordinate to bpy or phen, forming an *unbridged* ternary stacking adduct. Finally, in the bridged ternary complexes, where the metal ion coordinates to both ligands as in Cu(Arm)(Xao-H)$^+$, *no* intramolecular stacks can be formed for steric reasons.

With these reasonings in mind, the stability constants determined earlier[18,20] for the ternary M(bpy)(Xao-H)$^+$ and M(phen)(Xao-H)$^+$ complexes must be rejected as being too large:[18,20] with the exception of the Ni^{2+} systems, the $\Delta \log K_M$ values for all bpy or phen systems with Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, and Cu^{2+} are claimed to be positive;[18] for Ca^{2+}/bpy/(Xao-H)$^-$ even $\Delta \log K_{Ca} = +1.04$ is given. In this case, the error most probably occurred in the calculation procedure.

4. Sites of Metal Ion Coordination to Deprotonated Xanthosine. Purine nucleosides show a dichotomy between N-1 and N-7 for metal ion binding.[22] This is tentatively expressed for M(Xao-H)$^+$ complexes in the intramolecular equilibrium 10a. Release of the



(10a)

R = −ribose

proton from the H(N-1) site of neutral xanthosine leads to a negatively charged nitrogen, which should be an excellent binding site for many metal ions: The corresponding isomer, shown on the left side in equilibrium 10a, is designated as M(N-1/Xao-H)$^+$. On the right side in equilibrium 10a N-7 binding is indicated, a coordination type well-known for many purine derivatives,[5-11,13] and this species is now designated as M(N-7/Xao-H)$^+$, but its correct structure is difficult to assess. There is obviously the possibility of dislocating the negative charge in (Xao-H)$^-$ from N-1 toward O-6; this could favor chelate formation involving N-7 *and* O-6. However, so far no examples for such a direct chelation of 6-oxopurine derivatives are known;[7,22] there are indications for an indirect chelation, i.e. with a water between O-6 and the N-7-bound metal ion.[7] This view is also supported by X-ray studies of solid complexes.[5] This structural ambiguity is indicated by the thin dotted line between O-6 and M^{2+} in equilibrium 10a; evidently a similar water bridge could also be formed between O-6 and the N-1-bound metal ion. With these uncertainties in mind, which will be discussed somewhat further in section 6, the intramolecular equilibrium 10a is rewritten in a simplified way:

$$M(N\text{-}1/Xao\text{-}H)^+ \rightleftharpoons M(N\text{-}7/Xao\text{-}H)^+ \qquad (10b)$$

This equilibrium is quantified by the dimensionless equilibrium constant K_I:

$$K_I = [M(N\text{-}7/Xao\text{-}H)^+]/[M(N\text{-}1/Xao\text{-}H)^+] \qquad (11)$$

Hence, equilibrium 5a may be rewritten

$$M^{2+} + (Xao\text{-}H)^- \rightleftharpoons M(N\text{-}1/Xao\text{-}H)^+ \rightleftharpoons M(N\text{-}7/Xao\text{-}H)^+ \qquad (12a)$$

The experimentally accessible stability constant K_{exp} (cf. also eq 5b) is then given by eq 12b. The stability constants for the

$$K_{exp} = K^M{}_{M(Xao\text{-}H)} = \frac{[M(N\text{-}1/Xao\text{-}H)^+] + [M(N\text{-}7/Xao\text{-}H)^+]}{[M^{2+}][(Xao\text{-}H)^-]} \qquad (12b)$$

complexes with a sole N-1 or N-7 coordination are defined in eq 13 and 14, respectively. Combination of eq 12–14 gives eq 15,

$$K_{N\text{-}1} = [M(N\text{-}1/Xao\text{-}H)^+]/[M^{2+}][(Xao\text{-}H)^-] \qquad (13)$$

$$K_{N\text{-}7} = [M(N\text{-}7/Xao\text{-}H)^+]/[M^{2+}][(Xao\text{-}H)^-] \qquad (14)$$

and together with eq 11 also eq 16 and 17 are obtained.

$$K_{exp} = K_{N\text{-}1} + K_{N\text{-}7} \qquad (15)$$

$$K_{exp} = K_{N\text{-}1} + K_I K_{N\text{-}1} = K_{N\text{-}7} + \frac{K_{N\text{-}7}}{K_I} \qquad (16)$$

$$K_I = \frac{K_{exp}}{K_{N\text{-}1}} - 1 = \frac{K_{N\text{-}7}}{K_{exp} - K_{N\text{-}7}} \qquad (17)$$

Evidently values for either $K_{N\text{-}1}$ or $K_{N\text{-}7}$ have to be obtained to find an answer with regard to the position of equilibrium 10. As the acidity constant for the release of the proton from N-1 (eq 2) in xanthosine is known (Table I; section 1), an attempt is made in sections 6 and 7 to estimate values for $K_{N\text{-}1}$ (eq 13) and to use these for further evaluations; however, this evaluation is restricted to the 3d metal ions, as well as Zn^{2+} and Cd^{2+}, because the stability of the M(Xao-H)$^+$ complexes with the alkaline-earth ions is very low (Table II).

5. Correlations between Complex Stability and Ligand Basicity: Construction of Base Line Plots Quantifying the Metal Ion Affinity of N-1 and N-7. The existence of a linear relationship between log $K^M{}_{ML}$ and $pK^H{}_{HL}$ is well-known for many series of structurally related ligands (see ref 45 and references therein). This advance is due to Martin,[22] who showed that logarithms of the stability constants for Ni^{2+}, Cu^{2+}, and Zn^{2+} binding at pyridine or purine N-1 type nitrogens and imidazole or purine N-7 type nitrogens display a linear relationship with pK_a for the three metal ions and the two types of nitrogen ligating sites. On this basis the dichotomy of metal ion binding to N-1 versus that to N-7 in several purine nucleosides including adenosine was resolved for Ni^{2+}, Cu^{2+}, Zn^{2+},[46] and Pd(diethylenetriamine)$^{2+}$.[22,47] An uncertainty occurring with adenosine is that the experimentally determined stability constants are smaller than the values estimated from the log K versus pK_a plots.[11]

We believe that this apparent discrepancy is to a large part due to steric hindrance: The data for 2-methylpyridine[48] and other ortho-substituted pyridines[49] do not fit the mentioned correlations, in contrast to the case for meta- and para-substituted pyridines and pyridine itself.[22] CH_3 and NH_2 groups are comparable in size and therefore we consider the given N-1 reference lines[11,22,46] as not representative of the N-1 complexation tendency of adenosine, which carries an amino group next to N-1; i.e., the available reference lines provide estimates of log K for N-1 adenosine complexation that are too large. This view asks for the construction of base lines taking into account steric hindrance and also for a reevaluation of the metal ion distribution data[22,46] between N-1 and N-7 of adenosine; of course, this distribution also strongly depends on the validity of the pK_a value estimated for N-7 of neutral adenosine[46] (for further comments see section 9).

These reasonings on steric hindrance are confirmed by equilibrium constants determined now for cytidine systems (see Experimental Section: $I = 0.1$ ($NaNO_3$); 25 °C): $pK^H{}_{H(Cyd)} = 4.14 \pm 0.02$ (3σ), log $K^{Cu}{}_{Cu(Cyd)} = 1.56 \pm 0.06$, and log $K^{Ni}{}_{Ni(Cyd)} < 0.6$. The first two values agree excellently with literature data.[50,51] Cytidine carries, like adenosine, an amino group next to its

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Table III. Equilibrium Data Used for the Construction of log K versus pK_a Plots for N-7 or Imidazole-like and N-1 or Pyridine-like Binding Sites: Negative Logarithms of the Acidity Constants, pK_a, of the Ligands in Their Acidic Form and the Logarithms of the Stability Constants, log K, of the Corresponding Metal Ion Complexes

no.	ligand	acidic ligand form	pK_a	M^{2+} complex	log K Mn^{2+}	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}	Cd^{2+}
				N-7 or Imidazole-like Systems						
1	adenosine[a]	$H_2(Ado)^{2+}$	−1.56	$M(HAdo)^{3+}$				0.16	−0.89	
2	1-methylinosine[b]	$H(CH_3Ino)^+$	1.4	$M(CH_3Ino)^{2+}$			1.0	1.4	0.3	
3	inosine[b]	$H(Ino)^+$	1.4	$M(Ino)^{2+}$		0.8	1.1	1.3	0.31[c]	0.86[d]
4	guanosine[b]	$H(Guo)^+$	2.33	$M(Guo)^{2+}$		1.0	1.4	1.9	0.80[b,e]	1.17[e]
5	imidazole[f]	$H(Im)^+$	7.04	$M(Im)^{2+}$	1.25	2.40	3.03	4.21	2.51	2.71
6	1-methylimidazole[a]	$H(CH_3Im)^+$	7.39	$M(CH_3Im)^{2+}$		2.77[g]	3.44	4.61	2.98	
				N-1 or Pyridine-like Systems						
7	pyridine[h]	$H(py)^+$	5.26	$M(py)^{2+}$	0.14,[i] 0.24[j]	1.25	1.87	2.49	1.00	1.32[k]
8	4-(2-thienyl)pyridine[m]	H(th–py)$^+$	5.59	M(th–py)$^{2+}$			1.91	2.57	1.10	
9	4-methylpyridine[n]	$H(CH_3py)^+$	6.18	$M(CH_3py)^{2+}$		1.58[o]	2.11	2.88	1.40	1.57[p]
10	7-methylinosine[a]	$(CH_3Ino)^+$	6.57	$M(CH_3Ino\text{-}H)^{2+}$				3.04	1.43	
11	inosine[b]	Ino	8.7	$M(Ino\text{-}H)^{2+}$		2.1	2.8		2.4	
12	ammonia[q]	NH_4^+	9.38	$M(NH_3)^{2+}$	1.27	2.08	2.74	4.18	2.41	2.67

[a] I = 1.0 ($NaClO_4$); 21 °C.[46] [b] I = 1.0 ($NaClO_4$); 25 °C.[53] [c] In D_2O at I = 0.1–5 ($NaNO_3$); 27 °C.[28] [d] In D_2O at I = 0.1–3 ($NaNO_3$); 27 °C.[28] [e] In D_2O at I = 0.1–4 ($NaNO_3$); 27 °C.[28] [f] I = 0.1 ($NaNO_3$); 25 °C.[54] [g] I = 1.0 ($NaClO_4$); 21 °C: Kim, S.-H.; Martin, R. B. Unpublished results, 1982. Martin, R. B. Personal communication, March 1988. [h] I = 0.1 ($NaNO_3$); 25 °C.[55] [i] I = 0.5; 25 °C.[56] [j] I = 0.5; 25 °C,[57] [k] I = 0.1–1; 20–30 °C. Average of 14 values listed in ref 51 and 56–59 (see footnote *l*). [l] A view on the constants given in the literature shows that the influence of ionic strength and temperature is small. [m] I = 0.1 ($NaClO_4$); 25 °C.[48,49] The correct structure of the ligand is given in ref 49. [n] I = 0.1 ($NaClO_4$); 25 °C.[48] [o] I = 0.5–1.0; 25 °C; average from ref 51 and 56 (see footnote *l*). [p] I = 0.1–1.0; 25–30 °C; average from ref 51, 56, 57, and 59 (see footnote *l*). [q] I = 0.1 ($NaNO_3$); 25 °C for pK_a and $Mn(NH_3)^{2+}$ values.[54] The other values are literature averages (I = 0–2; 20–30 °C) taken from ref 54 (see footnote *l*).

pyridine-like nitrogen, and the given equilibrium data for this nucleoside do not fit on the previous base line plots for Cu^{2+} [46] and Ni^{2+};[11] i.e., the present stability constants are smaller than the previous ones.[46] In accordance with the present results are constants obtained earlier for 2-aminopyridine and Cu^{2+}:[52] these data do also *not* fit on the mentioned base line. Consequently, we have not included in the construction of the base line plots described below any cytidine or other 2-aminopyridine-like data. However, in agreement with previous conclusions,[22,46] we could not observe steric hindrance by a (smaller) keto oxygen in a position next to N-1 as occurs, for example, in inosine; it could be that this ortho oxygen is undergoing hydrogen bonding with a coordinated water molecule and that this diminishes or cancels its steric influence. This type of hydrogen bonding would also explain why steric hindrance in complexes of 2-aminopyridine is more pronounced (see also section 9) than in those of cytidine; the latter ligand has not only an amino group but also a keto oxygen in a position next to its coordinating pyridine-like N-3. Hence, for cytidine complexes one may expect that part of the steric effect of the 4-amino group is compensated for by hydrogen bonding of a metal ion coordinated water molecule to the 2-keto oxygen. Certainly, a final decision on this aspect requires more experimental data.

The procedure of Martin[11,22,46] for the construction of base line plots for the metal ion affinity of N-1 and N-7 of purine nucleotides is now extended to Mn^{2+}, Co^{2+}, and Cd^{2+}, and that for the complexation of Ni^{2+}, Cu^{2+}, and Zn^{2+} is repeated by taking into account the restrictions described above. The equilibrium constants used for the log K versus pK_a plots are summarized in Table III; as far as possible, we have selected constants[28,46,51,53–59] that were measured at (or close to) I = 0.1 and 25 °C. This latter aim is relatively well achieved for the N-1 or pyridine-like data, and these are mainly important for the evaluations in sections 6 and 7.

Table IV. Correlations between M^{2+} Complex Stability with Imidazole-like (N-7) or Pyridine-like (N-1) Ligands and Ligand Binding Site Basicity[a]

M^{2+}	m	b	R	SD[b]
	Regression Lines for Imidazole-like or N-7 Type Ligands			
Mn^{2+}	0.30[c]	−0.86		0.07[d]
Co^{2+}	0.315 ± 0.026	0.312 ± 0.135	0.993	0.057
Ni^{2+}	0.377 ± 0.020	0.519 ± 0.095	0.996	0.046
Cu^{2+}	0.499 ± 0.019	0.766 ± 0.084	0.997	0.053
Zn^{2+}	0.416 ± 0.016	−0.246 ± 0.069	0.997	0.045
Cd^{2+}	0.328 ± 0.020[e]	0.404 ± 0.070[e]	1.000	0.05[e]
	Regression Lines for Pyridine-like or N-1 Type Ligands			
Mn^{2+}	0.262 ± 0.021	−1.189 ± 0.145	0.997	0.029
Co^{2+}	0.204 ± 0.031	0.244 ± 0.236	0.977	0.045
Ni^{2+}	0.235 ± 0.025	0.633 ± 0.179	0.984	0.036
Cu^{2+}	0.415 ± 0.010	0.296 ± 0.065	0.999	0.013
Zn^{2+}	0.367 ± 0.025	−0.923 ± 0.178	0.991	0.034
Cd^{2+}	0.332 ± 0.013	−0.450 ± 0.094	0.999	0.015

[a] Slopes (m) and intercepts (b) are given for the straight base line plots of log K versus pK_a as calculated by the least-squares procedure from the experimental equilibrium constants of the systems given in Table III, together with the corresponding correlation coefficients (R). The column at the far right lists the standard deviations (SD) resulting from the differences between the experimental (Table III) and calculated (from the straight-line equations) log K values of the individual systems listed in Table III (I = 0.1–1 (see text and Table III); 25 °C). Straight-line equation: $y = mx + b$. x may represent the pK_a value of any N-7 or N-1 type ligand. The errors given with m and b correspond to one standard deviation (1σ). [b] These SD values times 2 or 3 are considered as reasonable error limits for any stability constant calculation in the pK_a range of the employed experimental data (see Table III or Figure 3). [c] This value is an estimate based on the other slopes in the table; together with the imidazole/Mn^{2+} data of Table III the N-7 type base line is thus defined. [d] Estimated error limit. [e] Due to the perfect fit of the experimental data (three points only; see Table III) on the calculated straight line, the calculated errors are very small, i.e. for m ±0.001, for b ±0.004, and for SD ±0.000; therefore, the estimates given above are considered as more realistic error limits.

The equilibrium data of Table III are plotted in Figure 3. The log K versus pK_a plots give two straight lines for each metal ion: one for the N-7 type and one for the N-1 type ligands. We were

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Table V. Logarithms of the Stability Constants for Metal Ion Coordination at N-1 or N-7 of N-1-Deprotonated Xanthosine, Together with the Intrinsic [N-1]/[N-7] Binding Ratios[a]

M^{2+} or H^+	log K_{exp} (eq 12b)[b]	log K_{N-1} (eq 13)[c]	log K_{N-7} (eq 14)[d]	log K_{N-1} − log K_{N-7}	$[M(N-1/Xao-H)^+]/[M(N-7/Xao-H)^+]$[e]
Mn^{2+}	0.84 ± 0.05	0.24 ± 0.09	0.71 ± 0.07	−0.47 ± 0.12	0.34 ± 0.09
Co^{2+}	1.65 ± 0.05	1.36 ± 0.14	1.34 ± 0.18	0.02 ± 0.23	1.0 ± 0.6
Ni^{2+}	2.09 ± 0.05	1.92 ± 0.11	1.60 ± 0.28	0.32 ± 0.30	2.1 ± 1.4
Cu^{2+}	2.58 ± 0.03	2.57 ± 0.04	<2[f]	>0.6[f]	>4[f]
Zn^{2+}	1.32 ± 0.02	1.08 ± 0.10	0.95 ± 0.14	0.13 ± 0.17	1.3 ± 0.5
Cd^{2+}	1.96 ± 0.05	1.37 ± 0.05	1.83 ± 0.07	−0.46 ± 0.09	0.35 ± 0.07
H^+	5.47 ± 0.03[g]	5.47 ± 0.03[g]	3.0 ± 0.5[h]	2.5 ± 0.5	~300[i]

[a] The errors given in columns 2 and 3 are *3 times* the standard errors; those in the other columns were calculated according to the error propagation method of Gauss. [b] Values of log $K^{M}_{M(Xao-H)}$ from Table II. [c] Calculated with pK^{H}_{Xao} = 5.47 and the N-1 base line equations of Table IV; the errors are 3 times the SD values given in Table IV. [d] Calculated via eq 15. [e] See text in section 6 and eq 18. [f] Calculated with the upper limit of 2.61 for log K_{exp} and the lower limit of 2.53 for log K_{N-1}. Direct calculation with the above values gives log K_{N-7} = 0.94 ± 2.16, log K_{N-1} − log K_{N-7} = 1.63 ± 2.16, and $[M(N-1/Xao-H)^+]/[M(N-7/Xao-H)^+]$ = 43 ± 212. [g] Value for pK^{H}_{Xao} from Table I. [h] Estimation; see text in section 6. [i] Ratio for [H(N-1) of $(Xao)^0$]/[H(N-7) of $H(Xao-H)^{\pm}$]; see text in section 6.

rather surprised to observe that the data for the ammonia systems of Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} fit within the error limits on the base lines for the pyridine-like or N-1 type ligands. This could be a result of mere chance, but it allowed us to construct corresponding base lines also for Mn^{2+} and Cd^{2+} (Figure 3). Similarly, regarding the use of the imidazole data (Table III) for the N-7 base lines, one might argue that a statistical correction should be introduced to account for the possibility that $H(Im)^+$ has two acidic protons, the release of one giving the coordinating neutral imidazole ligand. However, again the imidazole data fit well without such a correction on the N-7 base lines for Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}; therefore, we used the unaltered data also in constructing the base lines for Mn^{2+} and Cd^{2+} (Figure 3). The results of the least-squares calculations for the base lines are summarized in Table IV; the slopes of the base lines quantifying N-7 and N-1 type coordination for Ni^{2+}, Cu^{2+}, and Zn^{2+} are in excellent agreement with the slopes published by Kim and Martin.[46]

6. Metal Ion and Proton Affinity of N-1 and N-7 in Deprotonated Xanthosine. The equilibrium constants of the xanthosine systems (Tables I and II) are also inserted into Figure 3 (solid diamond points). The point for $Cu(Xao-H)^+$ fits exactly on the reference N-1 line; the point for $Ni(Xao-H)^+$ is also close to the N-1 line, whereas the point for $Mn(Xao-H)^+$ is significantly above; the data points for the other three xanthosine metal ion systems are in each case resting between the two individual N-1 and N-7 base lines. This appears to indicate, for example, that for Cu^{2+} N-1 binding is dominating and that Mn^{2+} coordinates to a significant extent at N-7.

A more quantitative evaluation is possible by applying eq 15: the values for K_{exp} are known (Table II), and values for K_{N-1} can now be calculated with pK^{H}_{Xao} = 5.47 (Table I) and the base line equations given in Table IV. The only unknown in eq 15 is K_{N-7}, and consequently this stability constant quantifying N-7 coordination to N-1 deprotonated xanthosine can now also be calculated. Knowledge of the stability constants K_{N-1} and K_{N-7} allows further calculation of the intrinsic [N-1]/[N-7] binding ratios; from eq 13 and 14 follows

$$\frac{[M(N-1/Xao-H)^+]}{[M(N-7/Xao-H)^+]} = \frac{K_{N-1}}{K_{N-7}} \qquad (18)$$

Hence, this ratio is best derived from the difference log K_{N-1} − log K_{N-7}. The results of these calculations are summarized in Table V.

There is one further aspect: knowledge of the metal-binding tendency of N-7 in $(Xao-H)^-$, i.e. of log K_{N-7} (Table V), now allows together with the N-7 type base line equations of Table IV for each metal ion calculation of a $pK_{a/N-7}$ value, the acidity constant of N-1-deprotonated but N-7-protonated zwitterionic xanthosine, $H(Xao-H)^{\pm}$. Obviously, no value can be obtained from the Cu^{2+} system (see Table V), and the Mn^{2+} system was not used due to the uncertainty of the corresponding N-7 base line (Table IV). The results for $pK^{H}_{H(Xao-H)}$ (=$pK_{a/N-7}$) values from the N-7 affinity of the other metal ions (in parentheses) are 3.26 ± 0.54 (Co^{2+}), 2.87 ± 0.38 (Ni^{2+}), 2.88 ± 0.23 (Zn^{2+}), and 4.35 ± 0.35 (Cd^{2+}); the given error limits correspond to one standard deviation (1σ). Clearly, the scatter of the data is large, which is not surprising considering the errors in log K_{N-7} and the relatively small slopes of the N-7 base lines, but the values overlap within 3σ and an estimate for $pK^{H}_{H(Xao-H)}$ may still be obtained. Calculation of the arithmetic mean using the number of data points on the N-7 base lines as weighting factors gives $pK^{H}_{H(Xao-H)}$ = 3.2 ± 0.4 (3σ); the mean without the Cd^{2+} value gives $pK^{H}_{H(Xao-H)}$ = 3.0 ± 0.1 (3σ). We consider as the best estimate with a conservative error limit $pK^{H}_{H(Xao-H)}$ = 3.0 ± 0.5. Combination of this value with pK^{H}_{Xao}

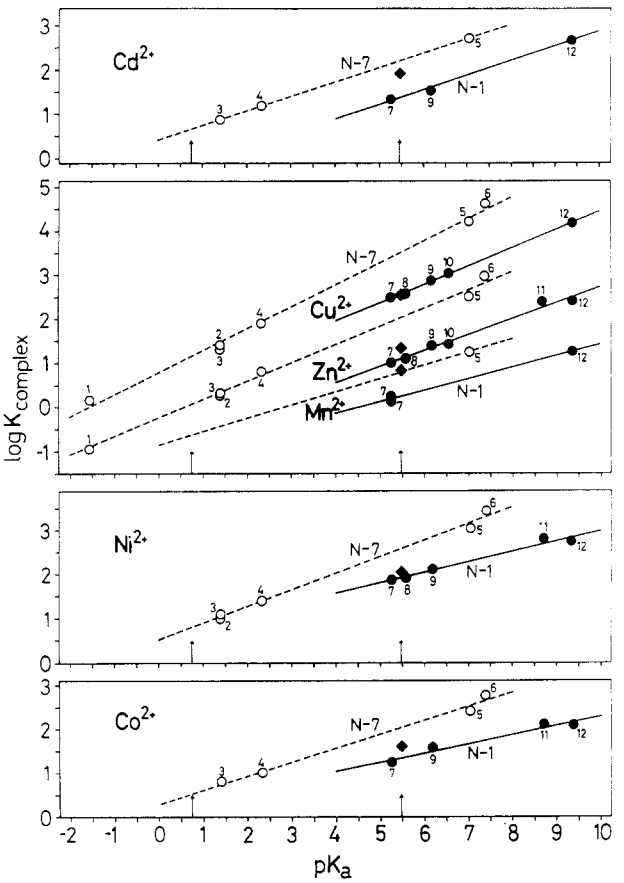

Figure 3. Relationship between log K and pK_a for the 1:1 complexes of Cd^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Ni^{2+}, and Co^{2+} (from top to bottom) with imidazole-like or N-7 type ligands (○, broken lines) and pyridine-like or N-1 type ligands (●, full lines). The least-squares lines are drawn through the data sets listed in Table III; the inserted numbers correspond to the ligand numbers in Table III. The resulting equations for the base lines (reference lines) are summarized in Table IV. The points due to the complexes formed between M^{2+} and $(Xao-H)^-$ are inserted for comparison (♦); the corresponding data are listed in Tables I and II. The two arrows placed on each pK_a axis correspond to the acidity constants of xanthosine; i.e., $pK^{H}_{H(Xao)}$ = 0.74 and pK^{H}_{Xao} = 5.47 (Table I).

Table VI. Comparison of the Metal Ion Affinity of N-1 and N-7 in N-1-Deprotonated Xanthosine, $(Xao\text{-}H)^-$: Percentages for the Two Isomeric Complexes in Equilibrium 10, Together with the Corresponding Intramolecular Equilibrium Constant K_I (Eq 11)[a]

M^{2+}	log Δ (eq 20)[b]	K_I (eq 11, 17, 19)	% M(N-7/Xao-H)$^+$ (eq 21)	% M(N-1/Xao-H)$^+$[c]
Mn^{2+}	0.60 ± 0.10	2.98 ± 0.94	75 ± 6	25
Co^{2+}	0.29 ± 0.15	0.95 ± 0.67	49 ± 18	51
Ni^{2+}	0.17 ± 0.12	0.48 ± 0.41	32 ± 19	68
Cu^{2+}	0.01 ± 0.05	~0 (<0.25)[d]	~0 (<20)[d]	~100 (>80)
Zn^{2+}	0.24 ± 0.10	0.74 ± 0.41	42 ± 14	58
Cd^{2+}	0.59 ± 0.07	2.89 ± 0.63	74 ± 4	26

[a] The error limits correspond to *3 times* the standard errors, calculated via the error propagation method of Gauss. [b] Calculated with eq 20 from the values listed in Table V. [c] These percentages follow from the difference from 100% with the values in column 4; the error limits are the same as in column 4. [d] The complete calculation based on log Δ gives K_I = 0.02 ± 0.12 and % Cu(N-7/Xao-H)$^+$ = 2 ± 11. The limits given in parentheses are calculated with log Δ < 0.1.

= 5.47 ± 0.03 (Table I) allows calculation of the intrinsic [N-1]/[N-7] binding ratio for the proton, i.e. of [H(N-1) of $(Xao)^0$]/[H(N-7) of H(Xao-H)$^\pm$]; this ratio is given in the bottom line of Table V.

The acidity constant $pK^H_{H(Xao\text{-}H)}$ = 3.0 ± 0.5 estimated for H(Xao-H)$^\pm$ is reasonable, as is seen from the following comparisons. Deprotonation of N-7 in $H(Xao)^+$ occurs with $pK^H_{H(Xao)}$ = 0.74 ± 0.06 (Table I); hence, the negative charge at N-1 in H(Xao-H)$^\pm$ leads to an interaction difference with the N-7 position of ΔpK_a = 2.3 ± 0.5. This value agrees excellently with corresponding N-1/N-7 interaction differences[46] for guanosine and inosine; i.e., ΔpK_a = 2.0 and 2.2, respectively.

The results in Table V show that especially the proton but also Cu^{2+} favor N-1 binding to the deprotonated $(Xao\text{-}H)^-$ species; N-7 binding is only dominating for the complexes with Mn^{2+} and Cd^{2+}. However, even here the experimentally measured stability constants (log K_{exp}) contain a noticeable contribution from N-1 coordination (log $K_{N\text{-}1}$).[60]

7. Position of the Intramolecular Equilibrium between N-1 and N-7 Binding in M(Xao-H)$^+$ Complexes. A closely related approach to the [N-1]/[N-7] ratio considered in eq 18 and the far right-hand column in Table V is based on the intramolecular equilibrium 10. Comparison of eq 11 and 18 makes the interrelation clear. The constants of Table V allow calculation of K_I (eq 17) via eq 19 and 20. Most instructive is the percentagewise quantification

$$K_I = 10^{\log \Delta} - 1 \tag{19}$$

$$\log \Delta = \log K_{exp} - \log K_{N\text{-}1} \tag{20}$$

of the isomeric complexes occurring in equilibrium 10; i.e., by eq 21.

$$\%\ [M(N\text{-}7/Xao\text{-}H)^+] = 100K_I/(1 + K_I) \tag{21}$$

The corresponding results are listed in Table VI.[60] Cu(Xao-H)$^+$ occurs largely as the N-1 isomer, while for the M(Xao-H)$^+$ complexes of Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+} a real distribution of the metal ion between the N-1 and N-7 sites is observed. The Irving–Williams sequence like order of the percentages for the isomers is interesting, indicating a systematic alteration of the relative affinities of the N-1 and N-7 sites toward the divalent 3d metal ions.

As the binary Cu(Xao-H)$^+$ complex forms mainly via N-1 coordination, the same property may be surmised for the two ternary complexes Cu(bpy)(Xao-H)$^+$ and Cu(phen)(Xao-H)$^+$. This interpretation agrees with Δ log K_{Cu} = −0.1 for both cases (cf. Table II). As the negative charge (i.e., the electron density) is probably somewhat less well localized on N-1 in $(XaO\text{-}H)^-$ as is the case with O donors such as phenolates or carboxylates, the observed Δ log K_{Cu} values agree with general experience: e.g., for coordination of the neutral 4-methylpyridine to $Cu(bpy)^{2+}$ one obtains Δ log K_{Cu} = −0.6[61] and for the corresponding coordination of acetate Δ log K_{Cu} = 0.0[39] (see also ref 36–38, 54, and 55).

8. Stability of M^{2+} Complexes with Neutral Xanthosine and Influence of pH on the N-1 versus N-7 Site Distribution. Neutral xanthosine (see Figure 1) offers N-7 as a binding site to metal ions. The stability of these complexes is defined by eq 4 in section 2. The low proton affinity of the N-7 site also suggests a low metal ion affinity, and indeed (see section 2) only estimates for log $K^M_{M(Xao)}$ of some systems could be obtained (Table II). However, the known acidity constant of N-7-protonated xanthosine, $pK^H_{H(Xao)}$ = 0.74 (Table I), together with the base line equations listed in Table IV for N-7 metal ion binding allows calculation of the corresponding stability constants. The measured (exp) and calculated (calc) stability constants agree satisfactorily; the "best" values for log $K^M_{M(Xao)}$ are listed in the fourth column of Table VII. These stability constants for the $M(Xao)^{2+}$ complexes follow the Irving–Williams sequence.

The existence of the complexes $M(Xao)^{2+}$ and M(Xao-H)$^+$ (eq 4 and 5) means that also equilibrium 22 must exist. In fact, the

$$M(Xao)^{2+} \rightleftharpoons M(Xao\text{-}H)^+ + H^+ \tag{22a}$$

$$K^H_{M(Xao)} = [H^+][M(Xao\text{-}H)^+]/[M(Xao)^{2+}] \tag{22b}$$

corresponding acidity constant may be calculated with eq 23.

$$pK^H_{M(Xao)} = pK^H_{Xao} + \log K^M_{M(Xao)} - \log K^M_{M(Xao\text{-}H)} \tag{23}$$

Comparison of these $pK^H_{M(Xao)}$ values in Table VII with pK^H_{Xao} = 5.47 (Table I) shows as expected a significant acidification of the H$^+$(N-1) site by the bound metal ions.

As the metal ion is coordinated in these $M(Xao)^{2+}$ complexes via N-7, it will change its binding position, at least in some cases, from N-7 to N-1 upon deprotonation of the N-1 site by giving the M(Xao-H)$^+$ species (discussed in sections 6 and 7). An equation derived by Martin[22] defines the crossover pH (=pH_c) for migration of a metal ion from N-7 to N-1 with increasing pH; when this equation is applied to the present situation, one obtains

$$pH_c = pK^H_{Xao} + \log K^M_{M(Xao)} - \log (K_{N\text{-}1} - K_{N\text{-}7}) \tag{24}$$

This equation defines the crossover pH for the situation where the amount of N-7-bound metal ion, which occurs in the $M(Xao)^{2+}$ and M(N-7/Xao-H)$^+$ species, equals that of the N-1-bound metal ion, which occurs only in M(N-1/Xao-H)$^+$; in other words, where R = 1 for the ratio given in eq 25.

$$R = \frac{[M(Xao)^{2+}] + [M(N\text{-}7/Xao\text{-}H)^+]}{[M(N\text{-}1/Xao\text{-}H)^+]} \tag{25}$$

It is evident that for Mn^{2+} and Cd^{2+} no pH_c value can be calculated, because N-7 binding is also dominating in the M(Xao-H)$^+$ complexes (see Table VI); i.e., a value of R = 1 is never reached. The results calculated for the crossover pH of the other metal ion systems with eq 24 and the equilibrium constants listed in Tables I, V, and VII (log $K^M_{M(Xao)}$ values) are given in the column at the right in Table VII. The pH_c values are below

(60) One should mention that the evaluations in sections 6 (Table V) and 7 (Table VI) might also be made with $pK^H_{H(Xao\text{-}H)}$ = 3.0 ± 0.5 and the N-7 base lines of Table IV. For comparison these results are given below for [M(N-7/Xao-H)$^+$] (%) (eq 10); use of $pK^H_{H(Xao\text{-}H)}$ = 2.5, *3.0*, and 3.5 gave the following (in this order) for the M(Xao-H)$^+$ systems: Mn^{2+} (11 ± 6/*16* ± *8*/22 ± 11), Co^{2+} (28 ± 12/*41* ± *17*/58 ± 24), Ni^{2+} (23 ± 8/*36* ± *12*/56 ± 19), Cu^{2+} (27 ± 10/*48* ± *18*/85 ± 32), Zn^{2+} (30 ± 10/*48* ± *16*/78 ± 25), Cd^{2+} (18 ± 5/*27* ± *8*/39 ± 12). Comparison of these results with those in the fourth column of Table VI shows that for most metal ions the agreement is excellent. The only real exceptions are the Mn^{2+} and Cd^{2+} systems: calculation with $pK^H_{H(Xao\text{-}H)}$ and the N-7 reference lines gives a lower amount of the N-7-coordinated isomer. We are not certain about the origin of this discrepancy, but most probably the reference lines are not well enough defined; for the Mn^{2+} N-7 reference line this seems quite obvious (see Tables III and IV). In any case, we believe that the evaluations given in sections 6 and 7 via pK^H_{Xao} = 5.47 ± 0.03 and the N-1 reference lines are more reliable, because for these data the error limits are significantly smaller (see also Table IV and text in section 5).

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Table VII. Stability Constants for the M^{2+} Complexes Formed with Neutral Xanthosine (Eq 4),[a] Acidity Constants for the $M(Xao)^{2+}$ Species (Eq 22), and Approximate Crossover pH Values (pH_c) from N-7 to N-1 Coordination (Eq 24)

M^{2+}	log $K^{M}_{M(Xao)}$ exp	log $K^{M}_{M(Xao)}$ calc	log $K^{M}_{M(Xao)}$ best	$pK^{H}_{M(Xao)}$	pH_c
Mn^{2+}		~−0.6 ± 0.2	−0.4 ± 0.4	4.2 ± 0.4	*b*
Co^{2+}	0.5 ± 0.2	0.55 ± 0.17	0.53 ± 0.13	4.35 ± 0.14	6.0
Ni^{2+}	0.7 ± 0.2	0.80 ± 0.14	0.77 ± 0.11	4.15 ± 0.12	4.6
Cu^{2+}	0.8 ± 0.3	1.14 ± 0.16	1.06 ± 0.14	3.95 ± 0.15	~4.0
Zn^{2+}		0.06 ± 0.14	0.06 ± 0.14	4.21 ± 0.14	5.0
Cd^{2+}	0.70 ± 0.12	0.65 ± 0.15	0.68 ± 0.09	4.19 ± 0.11	*b*
$Cu(bpy)^{2+}$	0.64 ± 0.18		0.64 ± 0.18	3.62 ± 0.18	*c*
$Cu(phen)^{2+}$	0.7 ± 0.3		0.7 ± 0.3	3.7 ± 0.3	*c*

[a] The experimental (exp) values for log $K^{M}_{M(Xao)}$ are from Table II, and the calculated (calc) ones are based on $pK^{H}_{H(Xao)} = 0.74$ and the N-7 base line equations of Table IV (see text). The fourth column contains the "best" constant; where two values exist, the weighted mean with $\pm 3\sigma$ is given. The value for $Mn(Xao)^{2+}$ is a rough estimate. [b] As N-7 binding is dominating, no crossover pH value exists; see text in section 8. [c] No values for log K_{N-1} and log K_{N-7} are known; therefore, pH_c cannot be calculated, but it is reasonable to assume that the value is close to 3.7.

Table VIII. Logarithms of the Stability Constants Measured for $M(Ado)^{2+}$ Complexes (log $K^{M}_{M(Ado)}$), as Well as Logarithms of the Stability Constants for Metal Ion Coordination at N-1 (log K_{N-1}) or N-7 (log K_{N-7}) of Neutral Adenosine, Together with the Intrinsic [N-1]/[N-7] Binding Ratios and the Percentage of the N-7-Coordinated Isomer, $M(N\text{-}7/Ado)^{2+}$, Formed in the Corresponding Intramolecular Equilibria[a]

M^{2+} or H^+	log $K^{M}_{M(Ado)}$	log K_{N-1}^{ortho} [b]	log K_{N-7} [c]	$[M(N\text{-}1/Ado)^{2+}]/[M(N\text{-}7/Ado)^{2+}]$	% $M(N\text{-}7/Ado)^{2+}$ [a]
Ni^{2+}	−0.17[d] 0.30 ± 0.13[e] 0.32[f]	0.28	0.59 (0.41/0.78)	0.49 (0.74/0.32)	67 (57/76)
Cu^{2+}	0.84[d] 0.70[g]	0.59	0.87 (0.62/1.12)	0.52 (0.93/0.30)	66 (52/77)
Zn^{2+}	−0.28[d] −0.3 ± 0.2[h]	−0.80	−0.16 (−0.37/0.05)	0.23 (0.37/0.14)	81 (73/88)
H^+	3.6[i]	3.6	0.2 ± 0.5[j]	~2500	~0.04

[a] The related data for $H(Ado)^+$ are given for comparison. The calculations were carried out analogously to those given in Tables V and VI for xanthosine; the percentages for the $M(N\text{-}1/Ado)^{2+}$ species follow from 100 − % $M(N\text{-}7/Ado)^{2+}$. [b] With $pK_a = 3.6$ and the N-1 base line equations in Table IV values for log K_{N-1} were calculated; from these values 1.2 log units were subtracted to account for the amino substituent in adenosine (see text in section 9). [c] Values for log K_{N-7} were calculated with the N-7 base line equations of Table IV and $pK^{H}_{H(N-7/Ado)} = 0.2$ (±0.5); the first value in parentheses is calculated with the lower pK_a limit of −0.3 and the second one with the upper limit of 0.7 (see also text in section 9). [d] Reference 63. [e] Reference 64. [f] Reference 65. [g] Reference 50. [h] Reference 28. [i] References 22 and 66. [j] See text in section 9.

$pK^{H}_{Xao} = 5.47$ for the Ni^{2+}, Cu^{2+}, and Zn^{2+} systems, as in these N-1 binding in $M(Xao\text{-}H)^+$ is favored. In the case of the Co^{2+} system, where the distribution of the metal ion between the N-1 and N-7 sites is nearly equal (Table VI), the crossover pH is only reached when the ligand has already largely lost its N-1 proton and is mainly present as $(Xao\text{-}H)^-$. In the physiological pH range (around 7) only $(Xao\text{-}H)^-$ coordinates to metal ions and for these conditions the site distributions given in Table VI apply.

9. Comments on the Metal Ion Affinity of the N-1 and N-7 Sites in Adenosine. Some problems regarding the distribution of metal ions between the N-1 and N-7 sites of adenosine have already been indicated in section 5. A further one becomes evident from a comparison of the stability constants in the fourth column of Table VII for the $M(Xao)^{2+}$ complexes, with the corresponding values in the second column of Table VIII for some $M(adenosine)^{2+}$ complexes: log $K^{M}_{M(Xao)}$ > log $K^{M}_{M(Ado)}$. Assuming for both complexes metal ion coordination to N-7, this observation is surprising because the corresponding acidity constant, $pK^{H}_{H(Xao)} = 0.74$ (Table I), is smaller than the value estimated for the release of a proton from N-7 in $H(Ado)^+$ with N-1 free, i.e. $pK^{H}_{H(N-7/Ado)} = 1.1$.[46,62] Even allowance of an error of ±0.2 log unit for the values of log $K^{M}_{M(Ado)}$ does not resolve the problem. As experience shows that complex stability increases with an increasing basicity of the binding site (e.g., Figure 3), we conclude that the estimate mentioned is too large. We conclude further that $pK^{H}_{H(Xao)}$ (=0.74) ≃ 0.8 ≥ $pK^{H}_{H(N-7/Ado)}$ and obtain in this way an upper limit for the latter pK_a value.

The stability constants of the $M(Ado)^{2+}$ complexes (Table VIII) may be used to calculate a $pK^{H}_{H(N-7/Ado)}$ value from the N-7 base line equations (Table IV). This procedure corresponds to that described for $pK^{H}_{H(Xao-H)}$ in section 6 and gives $pK^{H}_{H(N-7/Ado)}$ ≃ 0.0 ± 0.5, which might also rather be taken as an upper limit because complete N-7 coordination was assumed for the $M(Ado)^{2+}$ complexes in this estimation. A precise value for $pK^{H}_{H(N-7/Ado)}$ is evidently difficult to assess, but we believe it to be (in its extremes) between $pK_a = -0.5$ and +0.8 and estimate $pK^{H}_{H(N-7/Ado)} = 0.2 \pm 0.5$. As deprotonation at N-7 in 2-fold protonated adenosine occurs with[46] $pK^{H}_{H_2(Ado)} = -1.56$, an interaction difference of $\Delta pK_a = 1.8 \pm 0.5$ is obtained; this difference compares well with those given for xanthosine ($\Delta pK_a = 2.3 \pm 0.5$; section 6), inosine ($\Delta pK_a$ =2.2),[46] and guanosine ($\Delta pK_a = 2.0$).[46]

Only the adenosine systems with Ni^{2+}, Cu^{2+}, and Zn^{2+} are further evaluated because (i) they have been considered before[22,46] and (ii) their stability constants have been determined by at least two independent research groups (Table VIII).[63-66] Next, we need to estimate the size of the steric influence (see section 5) of an amino group next to an N-1 site; this is done by using 2-substituted pyridine derivatives. For each of the following five pyridine systems the first value is the pK_a, the second value the logarithm of the stability constant for M^{2+} binding (log K^{M}_{ML}), and the third value log K_{N-1} for metal ion N-1 binding as obtained from the mentioned pK_a and the base line equations of Table IV: 2-methylpyridine (Cu^{2+}),[48] 6.06, 1.3, 2.81; 2-methylpyridine (Ni^{2+}),[48] 6.06, ≤1, 2.06; 2-aminopyridine (Cu^{2+}),[52] 6.96, 1.71, 3.18; 2-amino-3-methylpyridine (Cu^{2+}),[52] 7.23, 1.91, 3.30; 2-phenylpyridine (Cu^{2+}),[49] 4.74, 0.7, 2.26. The difference log K_{N-1} − log K^{M}_{ML} equals, on average, 1.5 log units, in perfect agreement with the difference between two earlier published base lines valid for 3,4-substituted pyridines and 2-substituted pyridines.[49] To prevent overestimation of the steric effect, we deduce now only

(62) If in $M(Ado)^{2+}$ also N-1 coordination is taken into account, the result of the comparison is even worse.

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1.2 log units from calculated log K_{N-1} values to obtain log K_{N-1}^{ortho} constants.

By applying the results summarized in the last two paragraphs, we obtain the estimates given in the third column of Table VIII, which characterize the stability of N-1 type $M(Ado)^{2+}$ complexes. Column 4 lists the stabilities for N-7 type complexation by using $pK^{H}_{H(N-7/Ado)} = 0.2$; the first value in parentheses refers to the lower pK_a limit of −0.3 and the second one to the upper limit of 0.7. Now the ratios of N-1 over N-7 binding can be calculated (fifth column), as well as the percentages for metal ion coordination at N-7 in the $M(Ado)^{2+}$ complexes (last column); the lower and upper limits are given in parentheses. The corresponding data for the interaction between the proton and adenosine are listed at the bottom of the table. It may be noted that now the measured stability constants (considering ±0.2 log unit as the error range) are in much better accord with the calculated values for log K_{N-1} and log K_{N-7} than previously;[11,22,46] this also supports the present evaluation.

Though rather tentative with regard to the absolute size of the values, the data in Table VIII show that the metal ions coordinate preferably at the N-7 site of adenosine and *not* at the N-1 site as previously concluded.[22,46] Moreover, it should be noted that assumption of a larger $pK^{H}_{H(N-7/Ado)}$ value would further favor N-7 coordination; the same is true if a larger steric hindrance (i.e., >1.2 log units) due to the amino group next to the N-1 site is assumed. However, in agreement with the conclusions of Martin,[22] it is evident that the intramolecular equilibrium between N-7- and N-1-bonded isomers *exists* and that proton binding occurs overwhelmingly at N-1 of neutral adenosine.[22,46] More final conclusions can only be made after experiments have been carried out (for all metal ions) that allow construction of base lines for *o*-amino N-1 type ligands.

Caveat and Conclusions

The reevaluation of the metal ion binding tendency of N-1 versus that of N-7 in adenosine and the conclusion that the N-7 site is favored for Ni^{2+}, Cu^{2+}, and Zn^{2+} binding is important: now, the formation of macrochelates in complexes of adenine nucleotides[9,10,13] involving N-7 is expected. A more detailed reevaluation of the adenosine metal ion systems is clearly needed, but this should be based on more comprehensive experimental data. It is especially desirable to define the influence of ortho substituents on the N-1 reference lines more exactly; it appears that there are three such N-1 base lines. (i) One base line corresponds to unsubstituted N-1 or pyridine-like ligands; in fact, these reference lines are given in Table IV and they include also ligands with an *o*-carbonyl oxygen (Table III). The steric influence of this group is small (i.e., smaller than that of an amino group).[67] Indeed, the data for inosine and 7-methylinosine (Table III) fit well on the N-1 reference lines (Figure 3); hence, if there is any steric influence, it is offset by an oxo–metal ion interaction possibly via a water molecule (see section 5). (ii) There are certainly independent reference lines for N-1 type ligands with an *o*-amino group, as for 2-aminopyridine or adenosine; these base lines are expected (see section 9) to be about 1–1.5 log units, depending on the metal ion, below the reference lines of Table IV. (iii) It appears highly likely that in N-1 or pyridine-like ligands with an *o*-amino *and* an *o*-carbonyl group the steric effect of the amino group is partially offset by a "positive" oxo–metal ion interaction. A ligand of this type is cytidine (see also section 5), and there are indications that metal ions not only coordinate to this ligand via the pyridine-like N but also interact simultaneously with the carbonyl oxygen.[5,7] Consequently, these base lines should be situated between those of points i and ii; indeed, use of the cytidine results of section 5 give for Cu^{2+} and Ni^{2+} reference lines that are about 0.45 and (at least) 1 log unit, respectively, below those of Table IV.

The results obtained for xanthosine (sections 6–8) show that for xanthosine 5′-monophosphate (XMP) an overlap between N-1 deprotonation and the release of a proton from the phosphate group must be expected. In any case, in the neutral pH range XMP will exist mainly as a 3-fold negatively charged species; hence, formation of $M(X\text{-}H\cdot MP)^-$ complexes will occur. Moreover, in the physiological pH range $M(X\text{-}H\cdot MP)^-$ species will have a high probability for macrochelate formation.

There is one further aspect of general importance: the equations of the base lines quantifying the pyridine-like (or N-1) and imidazole-like (or N-7) coordination tendency of metal ions allows us to judge the reliability of published stability constants for nucleoside complexes. For example, stability constants published[68] for the $M(inosine\text{-}H)^+$ complexes of Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} are clearly too large if judged on the basis of the reference lines of Figure 3 or the equations listed in Table IV; in contrast, the corresponding constants for Co^{2+}, Ni^{2+}, and Zn^{2+} in ref 53 are in accordance with the expectations. Similarly, the stability constants published in ref 69 for $M(guanosine)^{2+}$ complexes have to be rejected, while those of ref 53 can be recommended. In addition, stability constants provided for $M(uridine\text{-}H)^+$ (cf. ref 70) and $M(cytidine)^{2+}$ (cf. ref 71) are also not reliable (with regard to the latter complexes see also section 5). It is important for researchers who apply stability constants of nucleoside–metal ion complexes, e.g. to evaluate the metal ion affinity of certain sites in DNA or RNA, to be aware of such pitfalls; otherwise, completely misleading conclusions may be reached. In such situations the base line equations of Table IV, together with the comments given above, may be used as a first guide in judging the validity of published stability data.

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